Distribution of recently fixed photosynthate in a switchgrass plant-soil system

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ABSTRACT

The use of switchgrass (*Panicum virgatum* L.) as an energy crop has gained great importance in past two decades due to its high biomass yields on marginal lands with low agricultural inputs and low maintenance requirements. Information on the allocation of photosynthetically fixed C in the switchgrass-soil system is important to understand the C flow and to quantify the sequestration of C in soils. The allocation of ^{13}C labeled photosynthates in shoot, root, soil, and in microbial biomass carbon (MBC) of rhizosphere and bulk soil of 45 days old, greenhouse grown-switchgrass was examined during 20 days ^{13}C-CO_2 pulse labeling period. The total ^{13}C recovered in the plant-soil system varied from 79% after 1 day to 42% after 20 days of labeling. After labeling, 54%, 40%, and 6% excess ^{13}C resided in shoot, root and soil, respectively on day 1; 27%, 61% and 11%, respectively on day 5 and 20%, 63% and 17%, respectively day 20 after labeling. The maximum incorporation of ^{13}C from roots into the MB of rhizosphere soil occurred within the first 24 h of labeling. The excess ^{13}C values of rhizosphere soil and rhizosphere MBC were significantly higher than excess ^{13}C values of bulk soil and the bulk soil MBC, respectively. The proportion of excess ^{13}C in soil as MBC declined from 92 to 15% in rhizosphere soil and from 79 to 18% in bulk soil, for 1 day and 20 days after labeling, respectively. The present study showed the effectiveness of ^{13}C labeling to examine the fate of recently photosynthesized C in soil-plant (switchgrass) system and dynamics of MBC.

Keywords: *Panicum virgatum* L.; ^{13}C pulse labeling; microbial biomass; rhizodeposition; carbon allocation; C sequestration

Switchgrass (*Panicum virgatum* L.) is a summer perennial C4 grass native to North American Tallgrass Prairie, primarily used for soil conservation, forage, urban landscaping, and wild life habitat (Keshwani and Chang 2009). Recently, there had been an increased interest to use switchgrass to produce biofuels (Keshwani and Chang 2009). The major benefits of switchgrass includes its wide geographical distribution, high biomass yields on marginal agricultural soils, natural resistance to many pests and plant diseases, and very low requirements for irrigation and fertilization (Keshwani and Chang 2009). Additionally, switchgrass can have 20–30 times greater C storage in soil than other crops (McLaughlin and Walsh 1998).

The flux of recently fixed carbon (C) from plant to soil is one of the least understood and poorly quantified parts of the C cycle (Cheng 1999, Kuzyakov and Domanski 2000). Plant roots secrete exudates, a variety of compounds that can account for up to 5 to 21% of photosynthetically fixed C transferred to the rhizosphere (Marschner 1995). Root exudates are important in regulating the structure and functioning of the microbial community in the rhizosphere (Walker et al. 2003). However, there are very few studies on the assimilation of photosynthetically fixed C into the rhizosphere microbial biomass, translocation below ground and exudation by rhizospheres (Norton et al. 1990, Kuzyakov and Domanski 2002, Butler et al. 2004, Yevdokimov et al. 2006). Understanding the process controlling C fluxes between plant roots, microbial biomass, soil and atmosphere is important for predicting and managing C sequestration in soils.

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To investigate the fate and partitioning of fixed C from CO_2 into rhizosphere and bulk soil, continuous or pulse-chase ^{13}C or ^{14}C labeling techniques are sensitive in quantifying labeled C in plant and soil fractions (Phillips and Fahey 2005, Yevdokimov 2006, Plain et al. 2009). For isotopic tracking studies ^{13}C is preferred over ^{14}C because of its lower discrimination of ^{13}C relative to ^{12}C during photosynthesis, greater safety, and lack of regulatory barriers for use and disposal (Svejcar et al. 1990).

So far, the partitioning and fate of photosynthetically fixed C in the switchgrass rhizosphere has not been studied. Therefore, the objective of this investigation was to determine the fate of photosythetically fixed C (^{13}C) in the switchgrass plant, roots, soil, and MBC of the rhizosphere.

MATERIAL AND METHODS

Soil and greenhouse experimental conditions. A Crosby silt loam (a Stagnic Luvisol in the FAO/WSR classification and a fine, mixed, mesic Aeric Ochraqualf in the USDA classification) soil was collected in July 2009 from the Waterman Dairy Farm of the Ohio State University, Columbus, USA. The initial total C and N contents of the soil were 1.32% and 0.086%, respectively, and pH was 7.2. Field moist soil passed through 4 mm sieve, homogenized and used for the experiment.

A greenhouse study was conducted using black plastic pots that were filled with 1250 g (dry weight basis) soil. Switchgrass (*Panicum virgatum* L., variety Cave in Rock) seeds were germinated on moist filter paper in petri plates. Three seedlings were transferred to each pot and thinned two plants per pot after transplanting. Plants were fertilized 0 and 15 days with a nutrient solution (100 mg/L) of 2.0, 0.44 and 1.65% of N, P, and K, respectively. Soil water content was maintained at two thirds field capacity by irrigating every 2–3 days intervals. In greenhouse the average daily temperature was 22–24°C, and the photoperiod was 12 h of illumination per day.

Pulse chase ^{13}C labeling. The plants were grown for 45 days and then labeled with $^{13}CO_2$ or $^{12}CO_2$. The labeling was done in two air tight chambers (as described below). One chamber had unlabeled ^{12}C-$NaHCO_3$ and the other with ^{13}C-$NaHCO_3$ (99.9 atom% ^{13}C; Cambridge isotope laboratory Inc., Andover, USA). To initiate $^{13}CO_2$ labeling, 4 mL of 1.5 mol/L HCl was added to a beaker containing 9.91 mg of ^{13}C as $NaHCO_3$ (per pot). Once the CO_2 concentration fell below 102 µmol/mol, HCl was added to an adjacent beaker containing 9.91 mg of ^{13}C as $NaHCO_3$ (second time). Similarly, HCl was added in third beaker (third time). At 1, 5, 10, and 20 days after labeling 3 replicate pots were destructively sampled. Soil (bulk and rhizosphere), stems, roots and microbial biomass were analyzed for total C and ^{13}C content.

The labeling was done in two chambers constructed of a wooden frame (0.61 m × 0.65 m × 0.90 m) with Teflon sheeting 'windows' on 5 out of 6 sides (excluding the bottom side). One 12VDC cooling fan (10 cm diameter, 4.2 W, 85 cfm) was installed to ensure proper circulation. Ice packs were placed inside the chamber to minimize excessive heating and to condense excess humidity. A hole was drilled at the bottom of the chamber and fitted with a rubber septum for use in labeling. Two holes were drilled on the top of the chamber with ports to fit tubing for CO_2 analyzer and CO_2 inside the chamber was continuously monitored with LI-6250 portable CO_2 analyzer (LI-COR Inc., Lincoln, USA). Supplemental lighting was placed to ensure optimal rates of photosynthesis during labeling.

Harvesting procedure. The root-soil systems were shaken in plastic container until approximately 80% of the initial soil was collected and this portion was considered as bulk soil. The remaining soil that was attached to the root system of plants was defined as rhizosphere soil. Root fragments remaining in the bulk and rhizosphere were carefully removed with help of forceps. Both types of soil samples were stored in plastic bags at –20°C till further use.

Plants and soil sub-samples were dried in oven at 50°C for 72 h. The dried plant samples were weighed and ground with a Wiley mill to pass a 100 mesh sieve and stored at 4°C. Subsamples of fresh soil were ground to pass a 100 mesh sieve with a mortar and pestle. These samples were used for determination of total C and $\delta^{13}C$.

Microbial biomass carbon (MBC). Soil was analyzed for microbial biomass carbon (MBC). MBC was extracted using fumigation-extraction procedure of Vance et al. (1987) as modified by Bruulsema and Duxbury (1996). Samples were analyzed for total C and $\delta^{13}C$ abundance as described below. An effective concentration (K_{EC}) factor of 0.45 was used to estimate MBC (Vance et al. 1987).

Isotopic analysis and calculations. Plant, soil and K_2SO_4 extracts were analyzed for total C and ^{13}C abundance with a CHN EA 1108 elemental analyzer (Carlo Erba Instruments, Lakewood, USA) coupled to a Delta V Advantage Isotopic Ratio Mass Spectrometer (IRMS) (Thermo Electron

Corporation, Bremen, Germany). By convention, ^{13}C abundance was expressed to Pee Dee Belemnite standard as either $\delta^{13}C$ or atom fraction ^{13}C excess.

The isotopic signal for C was expressed as $\delta^{13}C$ versus the international standard Pee Dee Belemite (PDB):

$$\delta^{13}C\ (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$$

Where: $\delta^{13}C$ is the parts per thousand, or per mil (‰), R_{sample} and $R_{standard}$ are the ^{13}C:^{12}C ratio of sample and standard (0.0112372), respectively.

Sample $\delta^{13}C$ (‰) was converted to milligrams C isotope using procedure described by Boutton (1999). The soil/plant $\delta^{13}C$ value first were converted to the absolute isotope ratio ($^{13}C/^{12}C$) of sample (R):

$$R_{sample} = {}^{13}C/{}^{12}C = [(\delta^{13}C/1000) + 1] \times R_{standard}$$

The fractional abundance (A) of ^{13}C relative to $^{13}C + {}^{12}C$ was then related to R_{sample} by the equation:

$$A = {}^{13}C/({}^{13}C + {}^{12}C) = R_{sample}/(R_{sample} + 1)$$

Fractional abundance and total C (mg) of sample were used to calculate ^{13}C of the sample:

$$mg^{13}C_{sample} = A \times \text{total C content of sample (mg)}$$

The enrichment level of the sample ($mg^{13}C_{sample}$) in excess of natural abundance ($mg^{13}C_{nl}$, non-labeled) was calculated as:

$$\text{Excess } mg^{13}C_{sample} = mg^{13}C_{sample} - mg^{13}C_{nl}$$

Excess $mg^{13}C$ of each pool (shoot, root and soil) was calculated as the product of excess $mg^{13}C_{sample}$ and pool mass. Recovery of ^{13}C was defined as the percentage of excess $mg^{13}C$ of each pool of the total $mg^{13}C$ added to the labeling chamber.

Statistical analysis. Analysis of variance (ANOVA) was used to evaluate time effects using the SAS statistical software package (SAS Institute, 1996). For all samples, rhizosphere and bulk soils were analyzed separately because they were not independent of each other. Difference between rhizosphere and bulk soils characteristics were analyzed using paired *t*-test. Significant differences are reported at the $P < 0.05$ level. Data are reported as mean of three replicates.

RESULTS

Plant biomass and carbon content. The total biomass of switchgrass increased from 3.73 to 4.70 g/pot during the chase period (Table 1). The increase in plant biomass was also reflected by an increase in total C accumulation, which increased from 1707 to 2347 mg/pot during the chase period (Table 1). The dry weight root and shoot biomass was similar. Initially, higher C accumulation was observed in shoot until 5 days after labeling. However, 10 days after labeling, higher C accumulation was noticed in roots.

Fixed ^{13}C-CO_2 recovery in plant and soil. The recovery of ^{13}C in shoot, and root biomass decreased during the chase period (Figure 1), which was significant in case of shoot only. One day after labeling, the ^{13}C recovery was highest in shoot biomass (43%), followed by root biomass (33%) and soil (4%). However, 5 days after labeling, root (29–27%) demonstrated the highest ^{13}C recovery until the end of labeling period (20 days) followed by shoot (13–8%) and soil (5–7%) (Figure 1). Total ^{13}C recovered in plant-soil system varied from 79% after 1 day to 42% after 20 days of labeling (Figure 1). Throughout the chase period, the $\delta^{13}C$ values in the shoot and root were significantly higher than the shoots and roots of the unlabeled control plants (shoots: –9.78‰, roots: –10.00‰ average of whole chase period). The average $\delta^{13}C$ value for shoot was 1306‰ 1 day after labeling and declined to 201‰ 20 days after labeling (data not shown). The dynamics of $\delta^{13}C$ values in the root followed a similar trend to the shoot, where average $\delta^{13}C$ of the root residue was 1099‰ after 1 day of labeling that declined to 572‰ after 20 days of labeling.

Pulse labeling resulted in excess ^{13}C content of 13 and 10 mg/pot for shoot and root, respectively, after 1 day of labeling that decreased to 3 and 8 mg/pot by the end of labeling period (20 days), respectively (data not shown). The decrease in

Table 1. The plant biomass and carbon content of switchgrass plant during labeling period

Days after labeling	Plant biomass (mg pot^{-1})			Carbon (mg pot^{-1})		
	shoot	root	total	shoot	root	total
1	1.93 (0.01)^{a}	1.80 (0.10)^{c}	3.73 (0.11)^{c}	904.54 (15.77)^{c}	802.36 (21.05)^{a}	1706.90 (55.88)^{c}
5	2.00 (0.09)^{a}	1.92 (0.13)^{bc}	3.92 (0.22)^{bc}	968.97 (17.34)^{bc}	907.37 (30.76)^{a}	1876.34 (64.83)^{bc}
10	2.08 (0.03)^{a}	2.29 (0.10)^{ab}	4.38 (0.11)^{ab}	1033.03 (12.28)^{ab}	1125.63 (28.41)^{a}	2158.66 (27.44)^{ab}
20	2.37 (0.12)^{a}	2.33 (0.13)^{a}	4.70 (0.24)^{a}	1093.85 (5.38)^{a}	1253.14 (40.04)^{a}	2346.99 (75.04)^{a}

Values in parenthesis are standard error. Values within a column with the same letters are not significantly different at $P < 0.05$

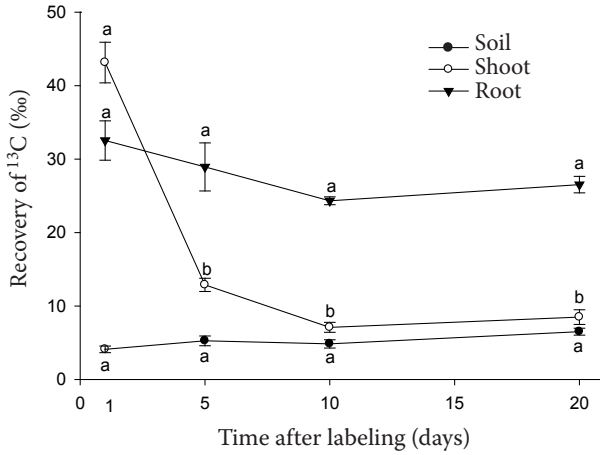

Figure 1. Recovery of ^{13}C in the plant-soil system (shoot, root and soil) of switchgrass during 20 days chase period. Error bars indicate standard error. Sampling points with the same lower case letters within a variable across sampling dates are not significantly different at $P < 0.05$

excess ^{13}C content of switchgrass was significant in case of shoot only.

Total C content of rhizosphere soil was higher than that of bulk soil, although, the differences in C content between rhizosphere and bulk soil were not significant (Figure 2). Throughout the chase period, the δ^{13}C values of both the rhizosphere and bulk soils were higher than the unlabeled planted control soils (δ^{13}C of unlabelled rhizosphere soil grown with switchgrass varied from –22.44 to –23.94‰ and bulk soil –29 to –30.10‰). The δ^{13}C values in the rhizosphere and bulk soil did not change significantly during the 20 days chase period. The δ^{13}C value in the rhizosphere soil declined steadily during the chase period, from an average of –9.49‰ to –20.00‰. Whereas, δ^{13}C of bulk soil increased from –21.63‰ to –16.67‰ during the chase period. Significant differences in excess ^{13}C were observed between rhizosphere and bulk soil on day 1 and day 5 of the labeling period (Figure 2). During the labeling period, rhizosphere soil on an average contained more than three times excess ^{13}C than in bulk soil.

^{13}C incorporation in microbial biomass. The MBC (labeled and unlabeled soil) in rhizosphere and bulk soil of switchgrass did not change significantly during the chase period (Figure 3). The MBC content of rhizosphere soil was significantly higher than in bulk soil throughout the chase period. The maximum incorporation of ^{13}C from roots into the MBC of rhizosphere soil occurred within the first 24 h of labeling. Later, excess

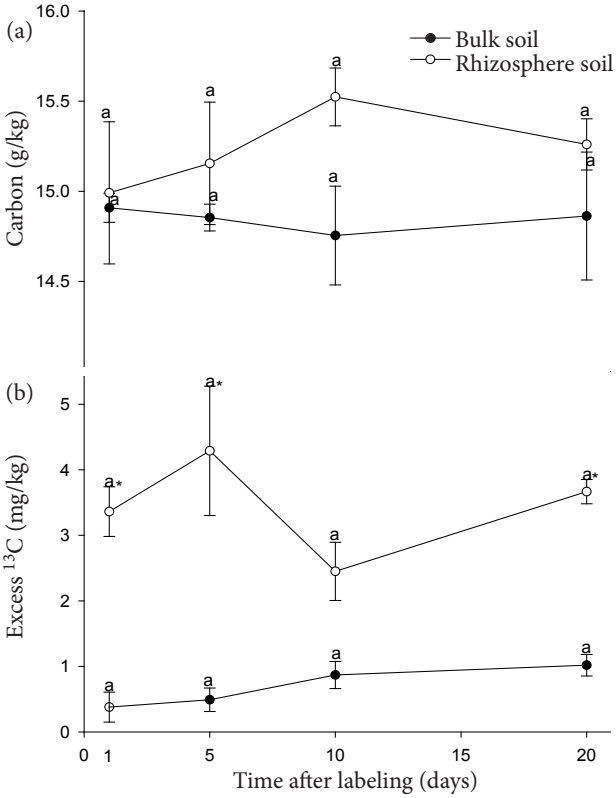

Figure 2. Total carbon (a) and excess ^{13}C content (b) in bulk and rhizosphere soil during 20 days chase period. Error bars indicate standard error. Sampling points with the same lower case letters within a variable across sampling dates are not significantly different at $P < 0.05$, *indicates a significant difference between bulk and rhizosphere soil at $P < 0.05$

^{13}C content of rhizosphere soil declined from 3.10 mg/kg after 1 day to 0.55 mg/kg after 20 days of labeling. In bulk soil there were no significant changes in microbial excess ^{13}C during the labeling period. There was a strong correlation (r = 0.78) between the excess ^{13}C of rhizosphere soil and bulk soil. The proportion of the ^{13}C that resided in the MBC pool of rhizosphere soil declined from 92% after 1 day of labeling to 30, 37 and 15% after 5, 10 and 20 days of labeling, respectively. Their respective decline in bulk soil was from 79% after 1 day of labeling to 46, 22 and 18% after 5, 10 and 20 days of labeling, respectively.

DISCUSSION

Carbon allocation. The C sink activity in roots is reported to be greater in the younger plants (Keith et al. 1986, Palta and Gregory 1997). Switchgrass has an extensive, deep root system and has been shown to produce more root biomass than corn (*Zea mays* L.) (Frank et al. 2004). The roots of young switchgrass plants in the present study demonstrated greater sink strength for C by storing more of the photosynthate in roots than in shoots (Figure 1). Similarly, Ma et al. (2001) found C storage in roots of switchgrass was 2.2 times higher than in shoots.

In the present study, there was less than 100% ^{13}C recovery, with 79% of the total $^{13}CO_2$ added recovered in the various plant and soil fractions during the labeling period. Similar observations were made on sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*), where 76 and 73% of assimilated ^{13}C was recovered after short-term ^{13}C pulse labeling, respectively (Phillips and Fahey 2005). This is likely due to respiration of shoot and roots, which was shown by Leake et al. (2006) even in a continuous flow saturated system. Gregory and Atwell (1991) found 15 to 25% of assimilated $^{14}CO_2$ was respired in 50-day old wheat or barley plants within 24 h after labeling began.

As expected we found the recovery of fixed CO_2 in the plant systems declined over time. This was from 79% of total ^{13}C added after 1 day of labeling to 42% by the end of experiment (20 days after labeling) (Figure 1). In a 2-day $^{13}CO_2$ pulse labeling of grassland vegetation, Ostle et al. (2000) within 24 h found a decline in ^{13}C assimilation by 76.4 and 61.65% in shoot and root, respectively. This rapid decline in pulse derived C in plant structural components can be attributed to losses of CO_2-C by root and shoot respiration and from root exudate-C as well as from dilution effect by uptake of unlabeled $^{12}CO_2$-C (Ostle et al. 2000).

The distribution of recovered ^{13}C showed that 54, 40 and 6% of ^{13}C was recovered after 1 day; 27, 61 and 11% after 5 days; and 20, 63 and 17%, after 20 days of labeling for shoots, roots, and soil, respectively (Figure 1). Butler et al. (2004) found an average of

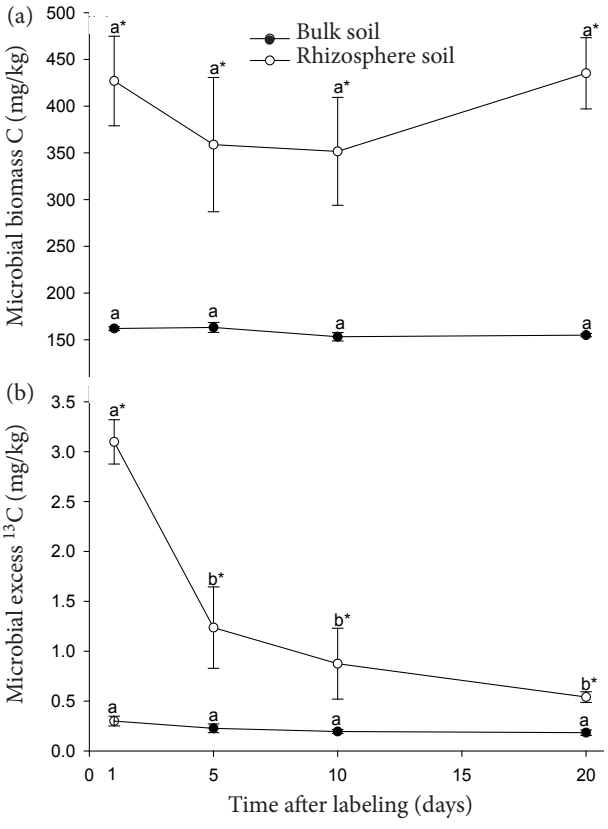

Figure 3. Microbial biomass carbon (a) and microbial excess ^{13}C in bulk and rhizosphere soil during 20 days chase period. Error bars indicate standard error. Sampling points with the same lower case letters within a variable across sampling dates are not significantly different at $P < 0.05$; *indicates a significant difference between bulk and rhizosphere soil at $P < 0.05$

70%, 20%, and 10% retained in aboveground biomass, roots, and soil, respectively, in a ryegrass (*Lolium multiflorum* Lam.) system after 8 days of exposure to $^{13}CO_2$. Our results show the importance of characterizing C fixation over the growing season as the distribution does change with time. Comparisons with other plant species the fate of fixed C, especially those that would be used for biofuels, is an important parameter in designing management systems for optimizing C sequestration.

The belowground translocation of assimilated C in our study ranged from 46 to 80% during the labeling period where majority of it was recovered in roots (Figure 1). This is similar to the amount of total assimilated ^{13}C distributed belowground in pasture plants (50 to 80%) found by Kuzyakov and Domanski (2000); where half was found in roots and about one-third lost as root and microbial respiration, with the remaining incorporated into MBC and soil organic matter. Whereas, Šantrůčková et al. (1999) when measuring the effect of doubling the atmospheric CO_2 levels (700 μmol/mol) on carbon budget in hydroponically grown (in sand) winter wheat plants, observed about 30% of daily assimilated C translocated to roots, 60% allocated into new biomass leaves, and 10% lost in respiration of leaves at night over the 5–34 days of averaged experimental life span. Half of C that was transported to roots was lost in root respiration and the remaining half in equal proportions was utilized for root biomass and release into the rhizosphere. Three-fifth of the C released in rhizosphere was lost in microbial respiration (Šantrůčková et al. 1999).

^{13}C-derived rhizodeposition. Several investigations on variety of crops species have shown that labeled CO_2 can be found in MBC within hours after the plant has been exposed to the C isotope (Cheng et al. 1993, Rattray et al. 1995, Lu et al. 2002). This indicates that recently assimilated C moves rapidly through plants to the roots and surrounding soil and is readily available to microorganisms. Time of maximum C incorporation into MBC after exposure to labeled CO_2 from studies done so far is from 1 to 5 days (Gregory and Atwell 1991, Kuzyakov et al. 2001, Lu et al. 2002). Our results with switchgrass are consistent with these studies where translocation of ^{13}C to rhizosphere MBC occurred rapidly, which was highest at 24 h after labeling and declined thereafter (Figure 3). Initially after 1 day, 92% of the rhizosphere soil and 79% of the bulk soil of the ^{13}C were was found in MBC. However this declined to 15% and 18%, respectively 20 days after labeling (Figure 3). This shows the importance of rhizosphere microorganisms in controlling the fate of root C exudates.

However, on a percentage basis of the amount of MBC is relatively low. In our study, between 2.7% and 5.1% of the plant-soil system ^{13}C was in the MBC pool on day 1 and 20, respectively (data not shown). This is in comparison to somewhat lower MB ^{13}C values reported for the rice rhizosphere 0.15 to 0.94% (Lu et al. 2002) and greater values of 1 to 5% for wheat and maize rhizosphere (Merckx et al. 1985, Liljeroth et al. 1990, Martin and Merckx 1992, Van Ginkel et al. 2000).

Significantly higher MBC and microbial excess ^{13}C was observed in rhizosphere soil than in bulk soil throughout the chase period (Figure 3). Higher MBC is generally observed in rhizosphere soil than in bulk soil (Butler et al. 2004). This is probably due to decreasing ratios of soluble-to-insoluble rhizodeposits with increasing distance from the roots (Whipps 1984).

The discussion above shows the importance of the rhizosphere MB in converting rhizo-deposited C to soil organic C in the bulk soil. This is further reinforced by the high correlation between ^{13}C levels in the rhizosphere soil and the bulk soil. And that MB-^{13}C declined in the bulk soil, while total ^{13}C in the soil slowly accumulated over the experimental period, further demonstrates movement of C through rhizosphere MB and into surrounding bulk soil.

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